

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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**July 17, 2003**

INVENTOR(S)

**John C. VOYTA, et al.**

EXAMINER

**Christine E. Foster**

GROUP ART UNIT

**1641**

TITLE OF APPLICATION

**SEQUENTIAL GENERATION OF MULTIPLE CHEMILUMINESCENT SIGNALS  
ON SOLID SUPPORTS**

Dear Sir:

I, John C. Voyta, hereby declare as follows:

1. I am a named inventor of the above captioned application.
2. I am currently an employee of Applied Biosystems. I have authored or co-authored numerous publications, including publications in the field of chemiluminescence.
3. I have read and reviewed the Official Action mailed January 9, 2006. I have also read and reviewed the references cited in the Official Action, including:
  - Cheek et al., "Chemiluminescence Detection for Hybridization Assays on the Flow-Through Chip, a Three-Dimensional Microchannel Biochip", Anal. Chem., 2001, 73, 5777-5783 (hereinafter referred to as "Cheek");
  - Akhavan-Tafti et al., "Chemluminescent Detection of DNA in Low- and Medium-Density Arrays, Clinical Chemistry 44, No. 9, 1998 (hereinafter referred to as "Akhavan-Tafti"); and
  - U.S. Patent No. 6,068,979 to Akhavan-Tafti (hereinafter referred to as "the '979 patent").

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4. It is my understanding that claims directed to assays conducted in the presence of a composition comprising a chemiluminescent enhancing material (e.g., Claim 2) have been rejected over Cheek, in view of the '979 patent as well as over the '979 patent in view of Akhavan-Tafti et al.

5. In order to demonstrate the effect of overcoating a high density array with a chemiluminescent enhancing material, the following experiments were performed. These experiments were conducted under my supervision or control.

6. High density arrays were spotted onto a film. The spots on the arrays were spaced approximately 100 microns center to center which corresponds to a density of 10,000  $\text{cm}^{-2}$ . The arrays were hybridized with a digoxigenin labeled cDNA sample prepared from liver polyA-mRNA (Stratagene, 0018) using a reverse transcriptase protocol (incorporated digoxigenin labeled dUTP, Enzo, 85996628). The arrays were hybridized overnight and subsequently processed for chemiluminescence detection.

7. After blocking to prevent nonspecific binding of enzyme labeled reagents, incubation with alkaline phosphatase labeled anti-digoxigenin and washing, arrays were incubated with either 0 mg/ml (FIG. 1A), 0.008 mg/ml (FIG. 1B), 0.04 mg/ml (FIG. 1C), 0.2 mg/ml (FIG. 1D), or 1.0 mg/ml (FIG. 1E) of TPQ polymer enhancer for 20 minutes prior to the addition of TFE-CDP-*Star*® substrate (2.5 mM in 0.1 M aminomethylpropanol, 1 mM  $\text{MgCl}_2$ , pH 9.5).

8. As shown in FIGS. 1A - 1E of the attached Exhibit, there is a substantial difference in chemiluminescent signal between the control (i.e., zero polymer used to overcoat the array) and the arrays overcoated with chemiluminescent enhancing material

at all of the concentrations tested. In particular, no chemiluminescent signal is visible in the absence of the TPQ enhancing polymer overcoat (FIG. 1A) whereas significant levels of detectable signal are present at all of the TPQ concentrations tested (FIGS. 1B-1E).

9. The images shown in FIGS. 1A - 1E are 25 second images obtained with a prototype ABI 1700 chip imager. CCD intensities of from 200 to 1000 are displayed.

10. This data clearly demonstrates the significant improvements in array performance realized by using a chemiluminescent enhancing material in a microarray format.

11. Additionally, the presence of the chemiluminescent enhancing material on the array surface has been found to restrict diffusion of the enzymatically deprotected dioxetane species.

12. Accordingly, the presence of a chemiluminescent enhancing material on the array surface provides an environment favorable to high quantum yield, spatially resolved chemiluminescent emissions.

13. Moreover, as set forth in a recent article by Cheek et al., “[c]hemiluminescence (CL) detection is seldom used in two-dimensional solid support microarray platforms because adequate sensitivity and spatial resolution is difficult to achieve” (Cheek et al., *Anal. Chem.*, 2001, 73, 5777-5783). In order to overcome these limitations, Cheek et al. employed a “flow-through chip” having three-dimensional ordered microchannels. In this manner, Cheek et al. were able to *physically confine* the probe and target into a reaction volume that is “small on the molecular diffusion length scale” (Cheek et al., sentence spanning pages 5778 and 5779).

14. In contrast, the present invention achieves high spatial resolutions without the use of glass microchannels or other physical means of confining the active chemiluminescent

species. As a result, conventional two-dimensional solid support materials can be used with chemiluminescence detection in microarray formats while still achieving high spatial resolution.

15. As evidenced by Cheek et al., these results would not have been expected.

I/We declare that all statements made herein of my/our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

5/18/06  
Date

John C. Voyta  
John C. Voyta



CANCELLED

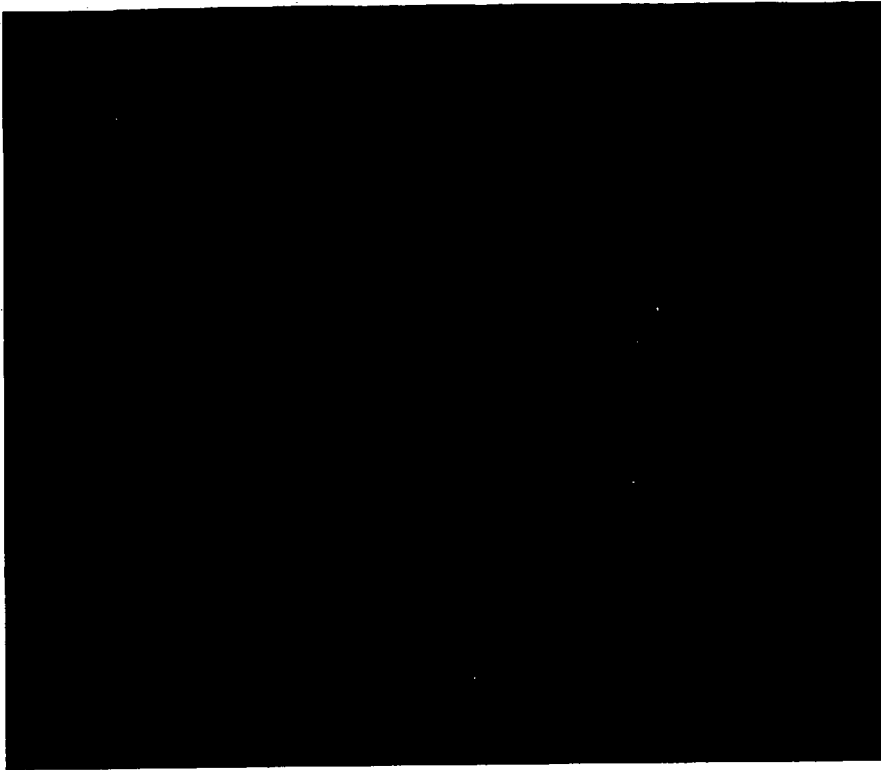


FIG. 1A

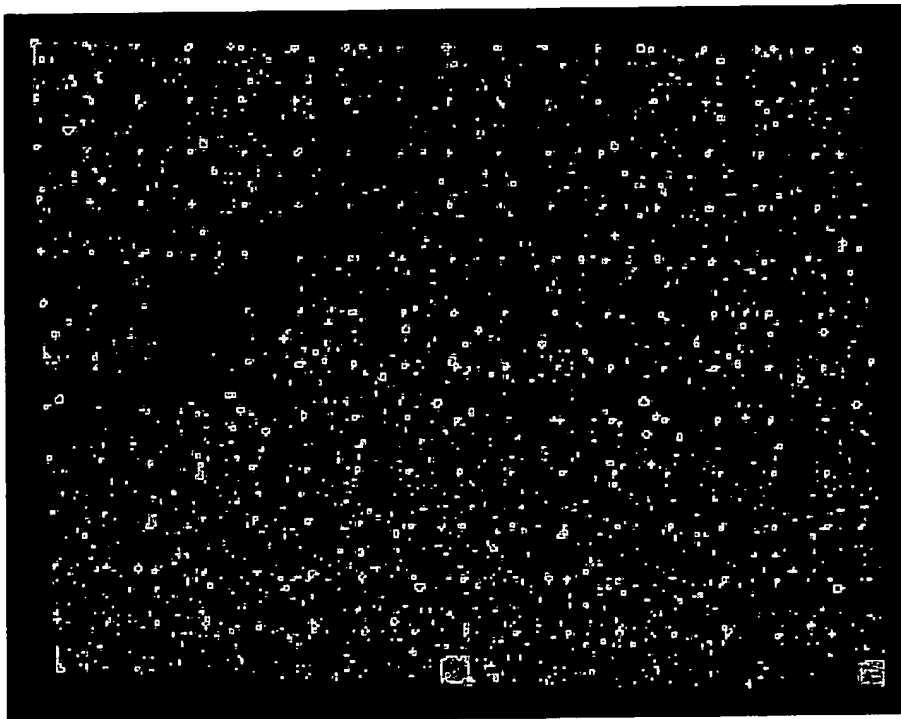


FIG. 1B

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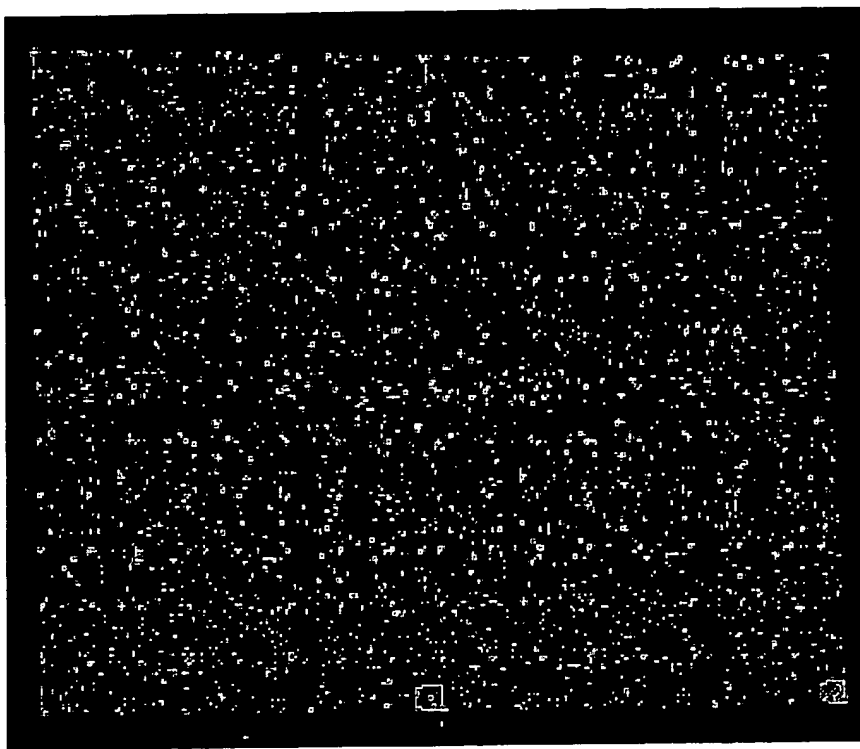
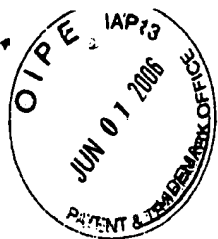


FIG 1C

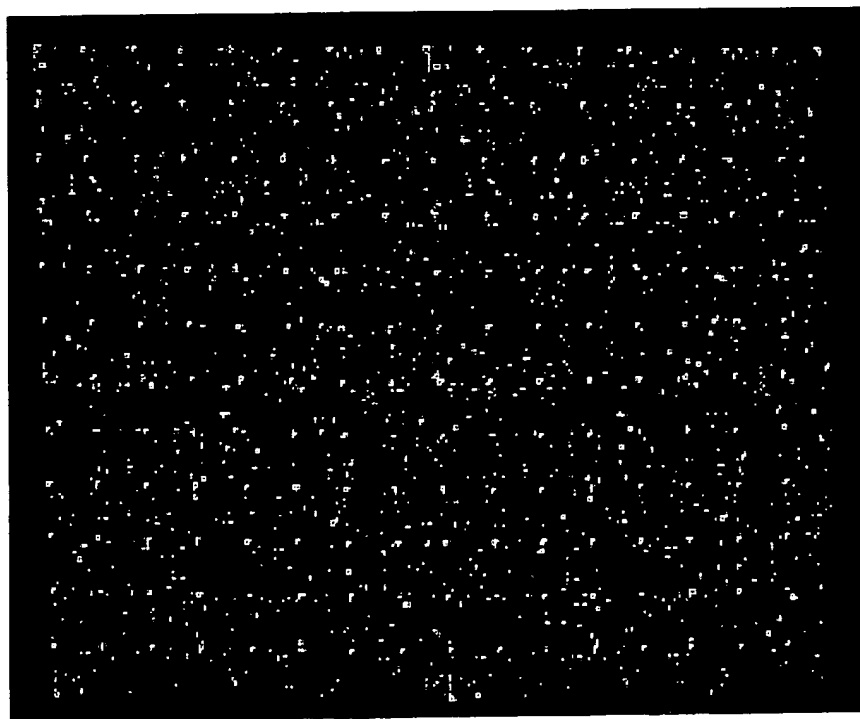


FIG 1D

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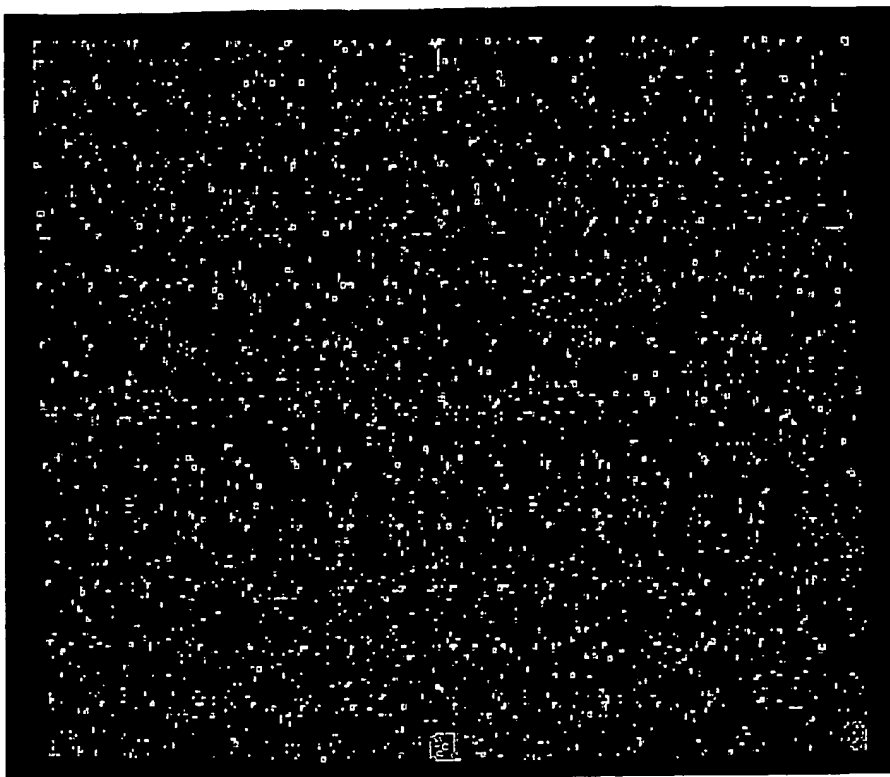
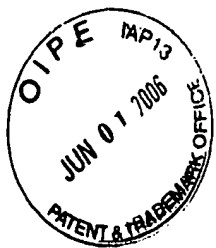


FIG 1E

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